IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Gauch et al.
Serial No.:	09/536,735
Filed:	March 28, 2000
Entitled:	ISOLATION OF NUCLEIC ACIDS ON SURFACES

ART UNIT: 1655

EXAMINER: B. Sisson

Attorney Docket No.: QGN-009.2 US

Assistant Commissioner for Patents
United States Patent and Trademark Office
Washington, D.C. 20231

RESPONSE TO AN OFFICE ACTION UNDER 37 C.F.R. § 1.111

Sir:

This paper is filed in response to the Office Action (Paper No. 8), dated June 20, 2001, in the above-identified application. Pursuant to 37 C.F.R. § 1.136(a), a Petition for a three-month extension in time is submitted concurrently herewith along with a check in payment of the fee under 37 C.F.R. § 1.17(a)(3).

Please amend the application as indicated below.

IN THE CLAIMS

Please amend Claims 1, 9, 14, and 65 as indicated below and on a separate sheet and in clean form pursuant to 37 C.F.R. § 1.121(c)(1)(i). A version of the amended claims with markings on a separate sheet pursuant to 37 C.F.R. § 1.121(c)(1)(ii) is also enclosed herewith at Tab A, and a complete set of pending claims, as amended herein, is provided pursuant to 37 C.F.R. § 1.121(c)(3) at Tab B.

Please delete Claims 57 and 76, without prejudice.

12/31/2001 CV0111 00000048 09536735
01 FC:117 920.00 OP

Amended Claims in Clean Form Pursuant to 37 C.F.R. § 1.121(c)(i)

1. (amended) A process for the isolation of nucleic acids from a sample including the following steps:

(a) applying at least one nucleic acid sample to a non-siliceous membrane;

(b) immobilizing the nucleic acids of the nucleic acid sample on the membrane in the presence of a compound selected from the group consisting of a salt of a metal and/or ammonium cation with a mineral acid, a salt of a mono or polybasic or polyfunctional organic acid with an alkaline or alkaline-earth metal, a hydroxy-functional compound of an aliphatic or acyclic saturated or unsaturated hydrocarbon, a phenol or polyphenol, a chaotropic agent, and combinations thereof, wherein the nucleic acids are reversibly immobilized on the membrane;

(c) releasing the immobilized nucleic acids from the membrane; and

(d) removing the released nucleic acids through the membrane, whereby the membrane is comprised of one or more materials selected from the group consisting of nylon, polysulfone, polyethersulfone, polycarbonate, polyacrylate, acrylic copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, polybenzimidazole, polyethylene-chlorotrifluoro-ethylene-copolymerisate, polyimide, polyphenylene sulfide, cellulose, cellulose-mix ester, cellulose nitrate, cellulose acetate, polyacrylnitrile, polyacrylnitril-copolymer, nitrocellulose, polypropylene and polyester.

9. (amended) A process for the isolation of nucleic acids from a sample comprising the following steps:

(a) applying at least one nucleic acid sample to a non-siliceous surface;

(b) immobilizing the nucleic acids of the nucleic acid sample on the surface in the presence of a compound selected from the group consisting of a salt of a metal and/or ammonium cation with a mineral acid, a salt of a mono or polybasic or polyfunctional organic acid with an alkaline or alkaline-earth metal, a hydroxy-functional compound of an aliphatic or acyclic saturated or unsaturated hydrocarbon, a phenol or polyphenol, a

chaotropic agent, and combinations thereof, wherein the nucleic acids are reversibly immobilized on the membrane;

(c) releasing the immobilized nucleic acids from the surface with an elution agent, characterized in that the release takes place at a temperature T, whereby $10°C \geq T \geq T_{S,EM}$, and $T_{S,EM}$ equals the freezing point of the elution agent.

14. (amended) A process for the isolation of nucleic acids from a sample comprising the following steps:

(a) adjusting a nucleic acid sample to binding conditions that permit reversible immobilization of the nucleic acids contained in the sample on a non-siliceous surface;

(b) applying the nucleic acids sample to the non-siliceous surface; and

(c) immobilizing the nucleic acids on the surface in the presence of a compound selected from the group consisting of a salt of a metal and/or ammonium cation with a mineral acid, a salt of a mono or polybasic or polyfunctional organic acid with an alkaline or alkaline-earth metal, a hydroxy-functional compound of an aliphatic or acyclic saturated or unsaturated hydrocarbon, a phenol or polyphenol, a chaotropic agent, and combinations thereof, wherein the nucleic acids are reversibly immobilized on the membrane, characterized in that, before and/or after adjusting the binding conditions there is a pre-treatment of the sample.

65. (amended) The process according to Claim 38, wherein washing steps are carried out using salt or buffer solutions selected from aqueous salt solutions of metal and/or ammonium cations with mineral acids, including alkaline halides, alkaline-earth halides, alkaline sulfates, alkaline-earth sulfates, alkaline phosphates, alkaline-earth phosphates, or mixtures thereof; aqueous solutions of salts of mono or polybasic or polyfunctional organic acids with alkaline or alkaline-earth metals, including sodium, potassium or magnesium salts of organic dicarboxylic acids including oxalic acid, malonic acid and succinic acid; aqueous solutions of sodium or potassium salts of a hydroxy or polyhydroxycarboxylic acid including citric acid; hydroxy-functional compounds of aliphatic or acyclic saturated or unsaturated hydrocarbons including C_1-C_5 alkanols and

aldites; phenols or polyphenols; one or more chaotropic agents including salts selected from the group of trichloracetates, thiocyanates, perchlorates, iodides, guanidinium hydrochloride, guanidinium isothiocyanate, and urea.

Bil cont

REMARKS

Applicants acknowledge election of Claims 1-20, 37-95,112-116, and 121-124 of Restriction Group I for prosecution in this application, but reserve the right to prosecute the withdrawn claims in a subsequent divisional application.

Furthermore, as the amendments made herein are for the purpose of directing coverage to particularly preferred embodiments of the invention or for the purpose of correcting errors that are typographical in nature, Applicants do not consider any of the amendments to be material to patentability of any prior claim. Applicants reserve the right to prosecute and to obtain the patent coverage to which they are entitled by statute for all embodiments of the invention.

Applicants have amended the claims to direct coverage in this application to a particularly preferred embodiment of the invention. In particular, Applicants have amended Claims 1, 9, and 14, and thereby also the claims depending therefrom, to cover a process for the isolation of nucleic acids from a sample in which the nucleic acids are *reversibly* immobilized on specific classes of *non-siliceous* membranes in the presence of specific classes of compounds so that the nucleic acids may be subsequently eluted from and through the membranes and thereby isolated for further use or manipulation, including cloning methods and various biochemical treatments. The claimed embodiment of the invention differs from all prior art procedures wherein nucleic acids are bound to a membrane or surface permanently, such as in Southern and Northern blotting, which do not permit subsequent isolation of the intact nucleic acids from the membrane in a purified form for subsequent use. According to Applicants' claimed invention, nucleic acid is reversibly immobilized on one of several specific non-siliceous membranes by applying the nucleic acid to the membrane in the presence of certain compounds selected from the group consisting of a salt of metal and/or ammonium cations with minerals, a salt of mono or polybasic or polyfunctional organic acids with alkaline or alkaline-earth metals, a phenol or polyphenol, a chaotropic agent, and combinations thereof.

Support for the amendments is found in the specification and claims (see, e.g., in the specification, p. 5, lines 17-28 (use of non-siliceous membranes); p. 6, lines 1-6 (elution through membrane); p. 17, line 1, to p. 18, line 11 (compounds for reversible immobilization on membranes); and original Claims 1, 46, 49, 54, 58, and 59). Accordingly, the amendments are

made to direct claim coverage to particularly preferred embodiment of the invention and add no new matter.

Applicants have canceled Claims 57 and 76, thereby making moot all rejections in the Office Action directed to these claims.

Applicants have amended Claim 65 to correct an inadvertent typographical error noticed by the Examiner. In particular, the amendment corrects the spelling of "or" in the phrase "phenols ir polyphenols". Accordingly, the amendment adds no new matter.

Entry of the above amendments is respectfully requested.

Claim Rejections Under 35 U.S.C. § 112, first paragraph

In the Office Action, the Examiner rejected Claims 51, 57, 58, and 72 as not enabled by the specification. The Examiner stated:

> "Claims 51, 57, 58, and 72 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or which it is most nearly connected, to make and/or use the invention. The specification has not been found to teach the conditions under which the various organic dicarboxylic acids recited in claim 51 are to be used; *i.e.,* 'oxalic acid, malonic acid, and/or succinic acid.' The specification also has not been found to teach the various members of the genus "aldite" and the conditions under which they are to be respectively used (claim 57). The specification has not been found to set forth the reaction conditions under which phenol or polyphenols are used to immobilize nucleic acids. And the specification has not been found to teach the conditions under which each of coating agents identified in claim 72 is to be utilized."

See, p. 4 of the Office Action (Paper No. 8, June 20, 2001). For the reasons expressed below, Applicants respectfully traverse the rejections.

As noted above, Claim 57 has been canceled, thereby making moot the Examiner's rejection of this claim.

With respect to the remaining claims, Applicants note that the specification clearly describes a process for isolating nucleic acids in a sample comprising reversibly immobilizing the nucleic acids to a non-siliceous membrane. For example, the specification describes the use of salts of polyfunctional organic acids, such as dicarboxylic acids, and alkaline or alkaline-earth

metals in the invention to immobilize nucleic acids to membranes according to the invention. According to the specification, such useful dicarboxylic acid compounds include oxalic, malonic, and succinic acids (see, e.g., p. 17, lines 7-11, of the specification). Guidance for use of such compounds in the claimed process of the invention is also provided in the Examples (see, Examples 19 and 20, p. 51, line 1, to p. 53, line 2, of the specification). Furthermore, the specification provides multiple examples of employing a chaotropic agent, such as guanidinium isothiocyanate, in various buffers used to reversibly immobilize nucleic acids in methods according to the invention (see, e.g., p. 17, lines 15-29, of the specification and Examples 1-31, pp. 30-63). The specification also provides recommended concentrations of various compounds useful in the invention (see, e.g., p. 17, line 22, to p. 18, line 5, of the specification). Accordingly, persons skilled in this art who read the specification will easily understand that the inventive feature of the claimed processes is the new discovery that certain compounds permit nucleic acids to be reversibly immobilized on non-siliceous membranes for subsequent elution and isolation through the membrane, without degradation, which isolated nucleic acids may then be further used or treated. The compounds and non-siliceous membranes useful in the methods of this invention are known to persons skilled in this art. Accordingly, such skilled practitioners are well aware that the compounds used in the process of the invention must be in amounts practical for suspending and working with nucleic acids and these membranes, as demonstrated by the various examples of reaction conditions for carrying out the claimed process in the specification. Furthermore, some optimization of procedures is both expected and routine by persons skilled in this art who typically must adjust to some degree most processes to a particular laboratory's plan or research situation. The inventive features of the invention are not embodied in the particular amounts of reagents used, and therefore recitation of particular non-inventive amounts would not serve to define Applicants' invention, as is the function of claims.

Applicants respectfully submit that the above-cited examples and explanations clearly show that the specification provides more than enough guidance to persons skilled in this art to carry out the preferred embodiments of the invention claimed in this application without any undue experimentation. Accordingly, the Examiner is requested to reconsider and withdraw the rejections under 35 U.S.C. § 112, first paragraph.

Rejection Under 35 U.S.C. § 112, second paragraph

In paragraph 6 of the Office Action, the Examiner made a rejection under 35 U.S.C. § 112, second paragraph, which recites Claims 54 and 57 as indefinite. The Examiner stated:

> "Claim 54 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.
>
> "Claim 57 is indefinite with respect to what constitutes an 'aldite'."

Applicants actually believe that this rejection is only meant to apply to Claim 57 because Claim 54 is directed to a specific embodiment of the methods of Claims 1, 9, or 19. However, since Applicants have canceled Claim 57, the rejection is now moot. If Applicants have misunderstood the rejection, they respectfully request clarification from the Examiner. Assuming Applicants have correctly understood the rejection as directed to Claim 57, the Examiner is respectfully requested to withdraw the rejection(s) under 35 U.S.C. § 112, second paragraph.

Rejections Under 35 U.S.C. § 102

In the Office Action, the Examiner has rejected a number of claims as anticipated under 35 U.S.C. § 102. In particular, the Examiner has rejected Claims 1-14, 16-20, 37, 46-48, 66-68, 70, 71, 73-76, 112-116, 124 as anticipated by U.S. Patent No. 5,561,064 ("Marquet"). The Examiner also considered Claims 9-20, 37-42, 46-50, 54, 55, 59-65, 76, and 112 to be anticipated by U.S. Patent 5,910,246 ("Walter") and Claims 9-20, 37-42, 46-49, 54-56, 59-65, 112, 114, 121, and 122 to be anticipated by U.S. Patent No. 5,081,028 ("Hofstetter"). Claims 1, 4, 6-8, 14, 16-20, 37-42, 46-49, 52, 53, 65, 73-75, 112, and 113 were rejected as anticipated by U.S. Patent No. 5,187,267 ("Comai"). For the reasons given below, Applicants respectfully traverse these rejections.

Applicants note that Claims 57 and 76 have been canceled thereby making moot any rejection directed to those claims.

The law regarding anticipation of a claim by a printed publication is clear. For anticipation under 35 U.S.C. § 102 by a printed publication, that publication must teach each and every element or aspect of the claimed invention. As explained in § 2131 of the Manual of Patent Examining Procedure (MPEP):

"TO ANTICIPATE A CLAIM, THE REFERENCE MUST TEACH EVERY ELEMENT OF THE CLAIM

" 'A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference.' *Verdegaal Bros. v. Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987). 'The identical invention must be shown in as complete detail as is contained in the . . . claim.' *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989)." (emphasis in original).

As noted above, the pending claims of this application are directed to a process for isolating nucleic acids in a sample comprising reversibly immobilizing the nucleic acids in the sample to non-siliceous membranes in the presence of certain classes of compounds (e.g., as recited in Claim 1) and subsequently removing the nucleic acids from non-siliceous membrane under certain specified conditions (e.g., as in Claim 9). A brief review of each of the patents cited by the Examiner clearly shows that none of the documents teaches or suggests Applicants' claimed invention.

Marquet

Marquet describes a method for purifying plasmid DNA without using cesium chloride/ethidium bromide ultracentrifugation gradients. The critical steps of the method described in Marquet are:

a) lysing cells containing the plasmid DNA;

b) removing cell debris and high molecular weight impurities to produce a clarified lysate containing plasmid DNA,

c) precipitating the plasmid DNA with a first precipitating agent, such as polyethylene glycol (PEG precipitation)

d) dissolving the precipitated plasmid DNA

e) precipitating the plasmid DNA again,

f) redissolving the plasmid DNA, and

g) applying the plasmid DNA to size exclusion or anion exchange chromatography to purify the plasmid DNA (see, e.g., abstract, Claims 1 and 3 of Marquet).

According to Marquet, the step of clarifying the lysate (step b, above) may be carried out using centrifugation and/or filtration (see, col. 8, lines 13-50 of Marquet). Another use of a filter is to physically retain and collect partially purified, precipitated plasmid DNA from solution (see, col. 8, lines 25-48 of Marquet). Such a collection step of precipitated plasmid DNA is based on simply retaining plasmid DNA precipitates from passing through the pores of the membrane (see, e.g., col. 8, lines 29-42 of Marquet), which is clearly not Applicants' invention, in which nucleic acid is reversibly immobilized on particular non-siliceous membranes such that the nucleic acid can subsequently be eluted from and through the membrane. Thus, Marquet does not teach each and every element of Claims 1-14, 16-20, 37, 46-48, 66-68, 70, 71, 73-75, 112-116, and 124 of this application.

The above explanation clearly shows that Marquet does not anticipate the claims under 35 U.S.C. § 102(b). Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

Walter

Walter describes a device similar to a centrifuge tube, but modified for the specific purpose of isolating nucleic acid. Walter also describes how to make and use the device (see, e.g., Fig. 1 and Example 1 of Walter). The device of Walter has fixed within it a nucleic acid binding glass material (e.g., glass fleece), an inlet, and an outlet (see, e.g., col. 2, line 12-col. 3, l 54-55 of Walter). Use of the non-siliceous membranes as described in Applicants' invention are not taught in the device and methods described by Walter. Furthermore, nowhere does Walter teach or suggest methods that employ agents to elute DNA immobilized on non-siliceous membranes at relatively low temperatures (e.g., at less than or equal to 10°C) as detailed in Claim 9 and claims depending therefrom (see, e.g., p. 6, line 25, to p. 7, line 8, of Applicants' specification). Clearly, Walter does not teach each and every element of pending Claims 9-20, 37-42, 46-50, 54, 55, 59-65, and 112. Accordingly, Walter does not anticipate these claims under 35 U.S.C. § 102(e).

In view of the above comments, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of the claims under 35 U.S.C. § 102(e).

Hofstetter

Hofstetter describes methods of producing polypeptides that bind IgE from recombinant cells. Hofstetter includes a description of a standard protocol that was employed for isolating

mRNA that comprises lysing eukaryotic cells with a lysis buffer containing guanidinium isothiocyanate and a subsequent oligo dT affinity chromatography step (col. 19, line 43-col. 20, line 27 of Hofstetter). The inventive feature of Applicants' claimed invention is not found in prior art oligo dT affinity chromatography, which involves complementary base-pairing between the poly A tail of mRNA and poly dT fixed to a surface or resin. Thus, the standard mRNA isolation method described in Hofstetter does not meet Applicants' claimed method of isolating nucleic acids comprising reversibly binding nucleic acid to non-siliceous membranes or the further feature of eluting the nucleic acids from the membrane at relatively low temperatures, such as less than or equal to 10°C according to Applicants' Claim 9 and claims depending therefrom (see, e.g., p. 6, line 25, to p. 7, line 8, of Applicants' specification). Clearly, Hofstetter does not teach each and every element of pending Claims 9-20, 37-42, 46-49, 54-56, 59-65, 112, 114, 121, and 122 and therefore does not anticipate the claimed invention under 35 U.S.C. § 102(b).

Comai

In the Office Action, the Examiner stated:

> "For purposes of examination, the claims have been interpreted as encompassing not only the above disclosed flow-[through] devices whereby DNA or RNA is/are isolated, but to also encompass traditional hybridization assays and the subsequent stripping of a probe from the hybridization membrane/filter.
>
> "Comai et al., column 13, first paragraph, disclose performing a traditional Southern blot whereby a nitrocellulose membrane has a capture sequence immobilized thereon and is subjected to the annealing of a complementary probe."

See, p. 7 of the Office Action. The inventive feature of Applicants' claimed method does not reside in prior methods of isolating nucleic acids that depends on complementary base pairing between the nucleic acids to be isolated and another complementary strand of nucleic acid permanently fixed to a membrane or surface as found in traditional Southern blot procedures. On the contrary, according to Applicants' invention nucleic acids are *reversibly* immobilized to a non-siliceous membrane in the presence of certain classes of compounds and then subsequently eluted from and through the non-siliceous membrane in a purer state and fit for further treatments or cloning. Thus, nowhere does the prior art Southern blot procedure, as described in Comai or any other document, teach or suggest Applicants' claimed method of isolating nucleic

acids in a sample comprising reversibly binding the nucleic acids to a non-siliceous membrane and subsequently eluting the nucleic acids from and through the non-siliceous membrane. Clearly, Comai fails to teach each and every element of Claims 1, 4, 6-8, 14, 16-20, 37-42, 46-49, 52, 53, 65, 73-75, 112, and 113 and therefore does not anticipate the claims under 35 U.S.C. § 102(b).

In view of all of the above comments, Applicants submit that it is clear that none of the references relied on by the Examiner anticipates the claims under 35 U.S.C. § 102. Accordingly, the Examiner is respectfully requested to reconsider and withdraw the rejections.

Rejections Under 35 U.S.C. § 103

In the Office Action, the Examiner rejected Claims 1, 9, 14, 19, 43-45, 66-69, 72, 77-95, and 123 under 35 U.S.C. § 103 as obvious over Marquet in combination with Walter and U.S. Patent No. 5,527,672 ("Mannsfield"). For the reasons provided below, Applicants respectfully traverse the rejection.

The standard for combining references to reject a claim as *prima facie* is also stated in the MPEP:

> **"FACT THAT REFERENCES CAN BE COMBINED OR MODIFIED IS NOT SUFFICIENT TO ESTABLISH *PRIMA FACIE* OBVIOUSNESS**
>
> "The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990)"

See, § 2142 MPEP (emphasis in original).

In addition, the above legal standard for rejecting claims as obvious over a combination of references was recently reiterated by the Court of Appeals for the Federal Circuit in *In re Kotzab*, 217 F.3d 1365, 55 USPQ2d 1313 (Fed. Cir. 2000). As the court in *Kotzab* noted:

> "A critical step in analyzing the patentability of claims pursuant to section 103(a) is casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only by the prior art references and the then-accepted wisdom in the field. See *Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. Close adherence to this methodology is especially important in cases where the very ease with which the invention can be

> understood may prompt one 'to fall victim to the insidious effect of a hindsight syndrome wherein that which only the invention taught is used against its teacher.' *Id.* (quoting *W.L. Gore & Assocs. Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 313 (Fed.Cir.1983)).
>
> "Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. *See In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed.Cir.1998): *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed.Cir.1984).
> . . .
>
> "The motivation, suggestion or teaching may come explicitly from statements in the prior art, the knowledge of one of ordinary skill in the art, or, in some cases the nature of the problem to be solved. *See Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617. In addition, the teaching, motivation or suggestion may be implicit from the prior art as a whole, rather than expressly stated in the references. *See WMS Gaming, Inc. v. International Game Tech.*, 184 F.3d 1339, 1355, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999). . . . Whether the Board relies on an express or an implicit showing, it must provide particular findings related thereto. *See Dembiczak*, 175 F.3d at 999, 50 USPQ2d at 1617."

See, *In re Kotzab*, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316-17 (Fed. Cir. 2000) (emphasis added).

As noted above, the motivation to combine may derive from many sources, however, the range of possible sources that may serve as evidence for a motivation to combine references "does not diminish the requirement for actual evidence. That is, the showing [of a motivation to combine] must be clear and particular." *In re Dembiczak*, 175 F.3d 994, 999, 50 USPQ2d 1614, 1617, 1999 WL 246572 (Fed. Cir. 1999) (emphasis added). Furthermore, "[b]road conclusory statements standing alone are not 'evidence.'" *Id.*

In considering the combination of references relied on by the Examiner to reject the claims as obvious, Applicants respectfully submit that nowhere in this record has the Examiner presented the required evidence of a motivation to combine the cited references to make Applicants' claimed methods *prima facie* obvious. However, even if the references are considered together the combination fails to suggest to persons of ordinary skill in this art Applicants' claimed invention.

Marquet and Walter are discussed above. Briefly, Marquet describes a method for purifying plasmid DNA that employs filtration to separate insoluble material and cell debris from cell lysates and may also employ filtration to retain and separate precipitates containing the plasmid DNA from solution. The final purification step of the method of Marquet employs separating plasmid DNA from other impurities by a size exclusion or anion exchange chromatograpy step (see, e.g., col. 3, lines 52-58; col. 4, lines 17-40; Claims 1 and 3 of Marquet). Nowhere does Marquet teach or suggest persons of ordinary skill in this art to reversibly immobilize nucleic acids to non-siliceous membranes using certain classes of compounds or the use of eluting agents at low temperatures according to Applicants' invention.

As noted above, Walter describes a device, similar to a centrifuge tube, and its use to isolate nucleic acid. The device (Fig. 1 of Walter) has a binding matrix, which Walter teaches should be a glass material, such as a "glass fleece" (see, e.g., col. 4, lines 54-55 of Walter). Walter includes a description of isolating nucleic acid with the aid of this device, which is an isolation with chaotropic buffer solution. The isolation of the nucleic acid is described in Examples 2 and 3 of Walter in which nucleic acid is bound on the glass fleece in the device (see, col. 5, lines12-25 of Walter). Such device and its use do not teach or suggest to persons of ordinary skill in the art Applicants' claimed methods of isolating nucleic acid comprising reversible immobilization of nucleic acid on non-siliceous membranes or the use eluting agents at relatively low temperatures. Furthermore, nowhere does Walter suggest that its device be combined with the method of Marquet to make Applicants' claimed methods.

However, even if combined, Marquet and Walter do not cure the deficiencies of one another to arrive at Applicants' claimed methods. In fact, Walter directs persons of ordinary skill in the art to bind DNA to glass materials, such as glass fleece, that are not used in Applicants' claimed invention, which employs non-siliceous membranes. Accordingly, when considered together Marquet and Walter do not provide persons of ordinary skill in this art with Applicants' claimed invention.

Mannsfield

Mannsfield describes a method of detecting a first molecule adsorbed to a water-treated hydrophobic membrane with a labeled second molecule that binds to the first molecule, all without the use of a blocking agent to prevent non-specific binding of the labeled second

molecule to the membrane (see, e.g., col. 3, lines 30-55 and col. 4, lines 12-37 of Mannsfield). According to Mannsfield:

> "The first molecule can be any molecule which has a complementary second molecule with which it can interact to form a complex molecule . . . The particular first molecule used is dependent upon the particular second molecule available for detection. The first and second molecules are complementary in that they form a complex together selectively. The second molecule does not interact with portions of the membrane which are hydrophobic and which are free of first molecules. The second molecule can be labeled with a radioactive label, a fluorescent label, an enzyme or any other moiety that reacts with a reagent so that it can be detected or itself can be the detected reagent"

See, col. 4, lines 38-53 of Mannsfield. Thus, Mannsfield describes a method of detecting a target molecule by its ability to specifically interact with a labeled second molecule, which is a binding partner of the target molecule. Nowhere does Mannsfield teach or suggest Applicants' claimed method for isolating nucleic acids in which the nucleic acids are reversibly immobilized on non-siliceous membranes to separate the nucleic acids from impurities and subsequently released from and collected through the membrane. Furthermore, nowhere does Mannsfield suggest that its method of detecting one binding partner with the other binding partner be combined with the methods of Marquet and/or Walter to arrive at Applicants' claimed invention.

Nevertheless, even if Mannsfield is combined with Marquet and Walter, the combination cannot provide the essential features of Applicants' claimed methods. The inventive feature of Applicants' methods of isolating nucleic acids neither depend on nor require, alone or in combination, filtration to separate insoluble impurities or DNA precipitates from solution as in Marquet, the use of glass fleece in a centrifuge tube device to bind nucleic acid as in Walter, and the detection of one member of a set of binding partners adsorbed to a membrane using a detectable form of the other member (as in Mannsfield). Clearly, no combination of the references can provide persons of ordinary skill in this art with the inventive features of Applicants' methods, such as reversibly immobilizing nucleic acids to the class of non-siliceous membranes in the presence of certain classes of compounds and/or the further feature of using eluting agents at relatively low, specified temperatures to obtain the nucleic acid. Only Applicants' disclosure provides the necessary teaching and guidance to place the claimed invention into the hands of persons of skill in this art and thereby advance the art.

Applicants submit that the above comments clearly show that not only is the combination of references relied on by the Examiner improper under 35 U.S.C. § 103, but even if the combination is made, it fails to make Applicants' claimed invention *prima facie* obvious to the hypothetical person of ordinary skill in this art. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection.

In view of all of the above comments, Applicants submit that the claims pending in this application are in proper form for allowance. Accordingly, the Examiner is respectfully requested to withdraw the rejections and pass Claims 1-20, 37-56, 58-75, 77-95, 112-116, and 121-124 to issue.

Respectfully submitted,

Thomas R. Berka

Thomas R. Berka, Ph.D. (Reg. No. 39,606)
Leon R. Yankwich (Reg. No. 30,237)
Attorneys for Applicants
YANKWICH & ASSOCIATES
130 Bishop Allen Drive
Cambridge, Massachusetts 02139
Tel.: (617) 491-4343
Fax: (617) 491-8801

CERTIFICATE OF MAILING BY EXPRESS MAIL

The undersigned hereby certifies that the items of correspondence referred to above are being deposited with the U.S. Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. §1.10, postage prepaid, in an envelope addressed to the Assistant Commissioner for Patents, United States Patent and Trademark Office, Washington, DC 20231 on the date indicated below:

Express Mail Label: 909963261 US

December 20, 2001
date of mailing and signature

Stephanie L. Leicht
Stephanie L. Leicht